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Full Length Research

Evaluation of the repellent activity of the oil extract of onion (*Allium cepa,* L.), against *Glossina palpalis gambiensis,* (v.)

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ABSTRACT: Transmission of African Trypanosomiasis is caused by the bites of the tsetse fly (*Glossina*) infected with microscopic parasites of the species *Trypanosoma brucei,* commonly in sub-Saharan Africa. The tsetse fly constitutes a major threat, and the disease poses a big socio-economic burden in sub-Saharan African countries. This study aimed to evaluate the repellent activity of the oil extract of fresh onion bulbs against *Glossina palpalis gambiensis.* The oil was extracted using cold maceration. The repellent activity of onion oil extract against laboratory-reared teneral *Glossina palpalis gambiensis,* was evaluated on the fore-arm skin of five human subjects under laboratory conditions. The data generated were subjected to statistical analysis using One-way analysis of variance. All graded concentrations of onion oil extract demonstrated significant repellent activity ($p<0.01$) by preventing tsetse flies from landing and probing on human skin, thereby highlighting its potential to reduce fly–human contact and offering a promising approach to lowering trypanosomiasis transmission and the associated public health burden.

Keywords: *Glossina palpalis gambiensis*, onion oil, public health concern, repellent activity, Trypanosomiasis.

INTRODUCTION

Human African Trypanosomiasis (HAT), also known as sleeping sickness, is a neglected tropical disease (NTD) caused by parasites of the species *Trypanosoma brucei.* The parasites are transmitted by tsetse flies (Genus: *Glossina*), which are found only in sub-Saharan Africa (Leak, 1998). Two different subspecies of pathogens cause two forms of the HAT: *Trypanosoma brucei gambiense* causes a slowly progressing African trypanosomiasis in western and central Africa, and *Trypanosoma brucei rhodesiense* causes a more acute African trypanosomiasis in eastern and southern Africa (Büscher *et al.,* 2017). Control efforts have reduced the number of annual cases, and in 2009, under 10,000 cases were reported, and the number of cases continues to drop. In 2020, fewer than 700 combined cases were reported by WHO (Franco *et al.,* 2022).

Glossina palpalis gambiensis (*G. p. gambiensis*) is one of the vectors of sleeping sickness, a Tsetse fly subspecies which is the chief agent of the parasite *Trypanosoma brucei gambiense* that causes the gambiense HAT (gHAT) throughout western and central Africa. The disease poses a big socio-economic burden in sub-Saharan African countries (Swallow 2000).

Biting of humans and livestock by infected tsetse flies is crucial in the transmission of trypanosomiasis. In view of its economic importance, research strategies aimed at breaking the cycle of transmission had been centred on interrupting the tsetse fly-human/animal interaction. Vector control strategies are therefore crucial in reducing the trypanosomiasis challenge, and the most effective control is to avoid contact with tsetse flies. Among the methods in use to achieve this is the use of synthetic insecticides. The use of synthetic insecticides in the suppression of tsetse flies is not ecologically friendly, as most synthetic insecticides are highly toxic to humans and non-targeted organisms, as well as harmful to aquatic life and terrestrial animals (Uilenberg 1998).

Phytochemicals are used by plants for self-defence against insects, pests, pathogens and environmental hazards. The mechanisms of action of these plant-derived chemicals are to produce medicinal, industrial, agricultural and commercial applications (Mazid *et al.,* 2011). Phytochemicals are responsible for the organoleptic properties of plants such as the smell of onion (*Allium cepa* Linnaeus), and are important in the development of environmentally friendly insecticides, pesticides and herbicides (Mazid *et al.,* 2011). This study was therefore initiated to evaluate the effect of *A. cepa* oil extract as a repellent to protect humans and livestock from tsetse fly bites and disease transmission, at the *Insectarium de Bobo-Dioulasso – Campagne d'Eradication de la Mouche Tsee-tsee et de la Trypanosomose (IBD-CETT), Bobo-Dioulasso, Burkina Faso.*

MATERIALS AND METHODS

The study was carried out at the Insectarium de Bobo-Dioulasso, Burkina Faso, located in the village of Darsalamy, 15 km from the town of Bobo-Dioulasso (IBD), Burkina Faso (Pagabeleguem *et al.*, 2021). The IBD is a facility that specialises in the mass-rearing of tsetse flies. It was built under the auspices of the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) initiative to satisfy the needs in sterile male tsetse flies for the PATTEC program in Burkina Faso and other countries infested by tsetse flies (Percoma *et al.,* 2018).

One thousand (1000) tsetse flies (*G. p. gambiensis*), from the IBD colony, were used for this research study in the experimental insectary arm of the mass rearing complex. The colony from where the experimental tsetse flies were obtained was fed *in vitro* on defibrinated and irradiated blood collected from slaughtered cows at the Bobo-Dioulasso abattoir, and maintained under the climatic conditions of 25 ±1°C and 75 ± 5% relative humidity. Each experimental batch of tsetse flies consisted of 30 starved laboratory-reared teneral *G. p. gambiensis* held in a Roubaud cage (13.5 × 8 × 4.5 cm) and introduced into each experimental cage. The tsetse fly experimental cages used had some modifications to the WHO (2009) recommended experimental cage, with frames of size 45 cm per side with a clear transparent netting on all four sides for viewing, and a fabric sleeve for access on the front.

Fresh onion bulbs

Fresh onion bulbs were bought at Yankura market in Kano, Kano State, Nigeria. The fresh onion bulbs were cultivated at Jaba town, Fagge Local Government Area of Kano State. Identification and authentication of the fresh onion bulbs were done at the Herbarium of the Department of Biological Sciences, Faculty of Science, Nigerian Defence Academy, Kaduna, with Herbarium voucher reference number NDA/BIOH/2022/11. The fresh onion bulbs were taken to the laboratory of the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmaceutical Sciences, College of Natural and Pharmaceutical Sciences, Bayero University, Kano, for onion oil extraction by the cold maceration method using n-hexane (Zhang *et al.* 2007). The extracted onion oil was highly volatile, and as such was preserved in a dark bottle with tight-fitting lid and kept in the refrigerator at 4°C until used.

Volunteers and inclusion criteria

Testing of repellents on human subjects yields results that are relevant to the actual conditions of use (Harrington et al., 2020). Five (5) apparently healthy adult male human test volunteers were used as human subjects in this study. Test volunteers included in this test were people who did not smoke cigarettes or who did not use tobacco, and did not have wounds or irritable skin. After selection, they were instructed to avoid the use of odorous substances such as fragranced soaps, fragranced creams, perfumes and repellent products on their bodies for 12 hours before and during testing because such factors may alter a person's attractiveness to tsetse flies.

Ethical clearance and signed informed consent

Ethical approval to conduct this study was obtained from the Institutional Ethics Committee for Health Sciences Research, Ministry of Health, Bobo-Dioulasso, Burkina Faso, with reference number N/Ref. A042-2022/CEIRES/IRSS. Written informed consents were obtained from the five human subjects before the commencement of the tests.

Experimental procedures

Allergy screening for test volunteers

Allergy screening was carried out for test volunteers using a test dose of 0.25 ml of 100% concentration of onion oil, applied to an area of 4 cm^2 of each test volunteer's forearm skin. Test volunteers with allergy reactions such as inflammation or skin irritation were excluded from participating in the study. All test volunteers were adult males and indigenes of the study area, Bobo-Dioulasso, Burkina Faso, which is an old endemic area of the disease.

Repellent activity

A 600 cm^2 area of skin on the left fore-arm (between the hand and elbow) of each test volunteer was washed with unscented soap, rinsed with clean water, and allowed to dry prior to testing. One thousand (1000) laboratory-reared teneral *G. p. gambiensis* starved for 48 hours were used to evaluate the repellent activities of graded concentrations of 50%, 75%, 87.5% onion oil extract reconstituted in 100% BETIS® Extra Virgin olive oil, and 100% concentration of onion oil extract (WHO, 2009). The different concentrations of the onion oil were applied separately on the fore-arm skin of test volunteers for a duration of 1 hour prior to exposure to these tsetse flies in different experimental cages, each cage containing thirty (30) starved laboratory-reared teneral *G. p. gambiensis*, in the laboratory, under conditions of normal room temperature and daylight. At each hour, each volunteer introduced his hand into the experimental cage containing 30 starved tsetse flies for a period of 5 minutes only. The number of tsetse flies that landed and/or commenced to probe the skin was counted each time by the test volunteer and recorded. This process was repeated two more times in each hour, at an interval of 20 minutes, with 5minutes exposure duration for each concentration. The test duration was 5 hours.

Determination of protection provided by onion oil extract against G. p. gambiensis landing and/or probing on human skin

The protection provided by the graded concentrations of onion oil extract against *G. p. gambiensis* landing and/or probing on human skin was determined by the number of tsetse flies landing and/or probing on the skin, counted each time by the test volunteers. The protection provided by the onion oil against *G. p. gambiensis* landing and/or probing on human skin, was calculated using a timer watch, as the number of hours that elapsed between the time when the fore-arms treated with the various concentrations of onion oil extract were inserted into different experimental cages each containing 30 starved tsetse flies, and the first tsetse fly that commenced to probe the skin. Data generated were expressed as mean $\pm$ standard error of the mean (mean $\pm$ SEM). The data were subjected to statistical analysis using One-way Analysis of Variance (ANOVA). Graphpad Prism version 8.2 for Windows (Graphpad Software, San Diego, California, USA) was used to analyse all the data. Values of $p < 0.01$ were considered significant.

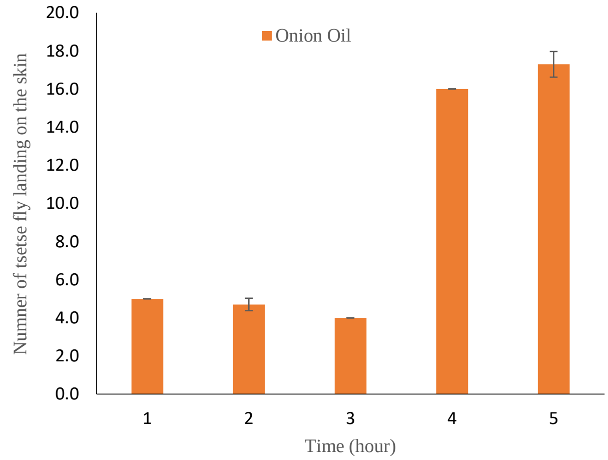

Figure 1a. Showing the mean number of *Glossina palpalis gambiensis* landing on skin after application of 50% onion oil ($p<0.01$).

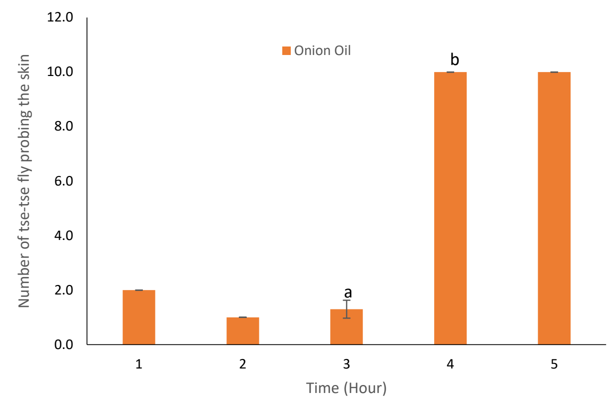

Figure 1b. Showing the mean number of *Glossina palpalis gambiensis* probing on skin after application of 50% onion oil. Means with different superscript letters ([a-b]) in different bars differ significantly ($p<0.01$).

RESULTS

Mean number of *Glossina palpalis gambiensis* landing, and probing on skin post-application of 50% and 75% concentrations of onion oil extract

At the 3rd hour post-application of 50% concentration of onion oil extract on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, onion oil performed significantly ($p < 0.01$) well at repelling *G. p. gambiensis* from landing with only 4.0$\pm$0.00 mean number of *G. p. gambiensis* landing on skin (Figure 1a).

At the 3rd hour post-application of 50% concentration of onion oil on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, onion oil performed significantly ($p < 0.01$) well at repelling *G. p. gambiensis* from probing with only 1.3$\pm$0.33 mean number of *G. p. gambiensis* probing on skin (Figure 1b).

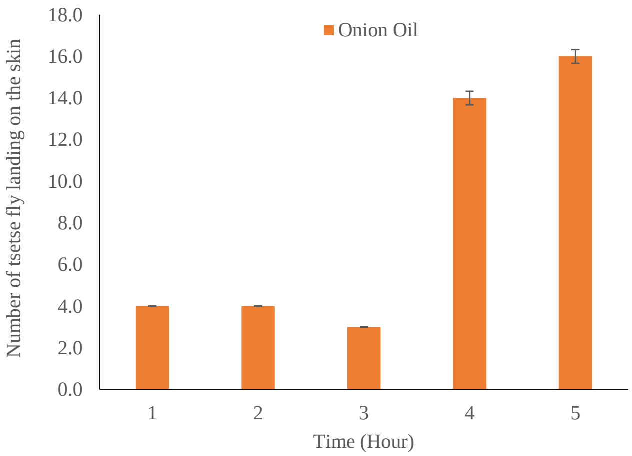

Figure 2a. Showing the mean number of *Glossina palpalis gambiensis* landing on skin after application of 75% onion oil (p<0.01).

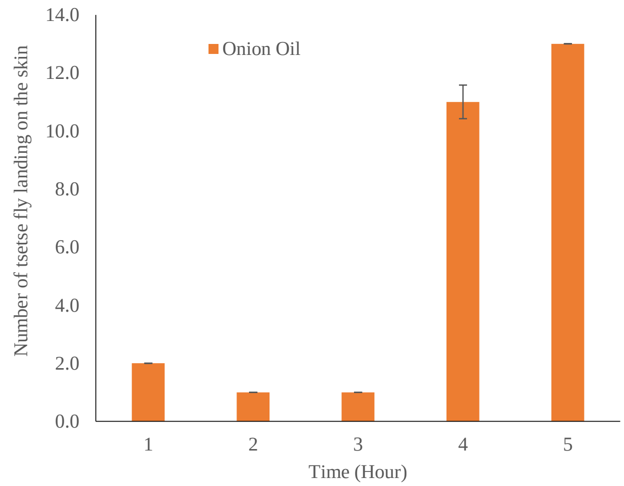

Figure 3a. Showing the mean number of *Glossina palpalis gambiensis* landing on skin after application of 87.5% onion oil (p<0.01).

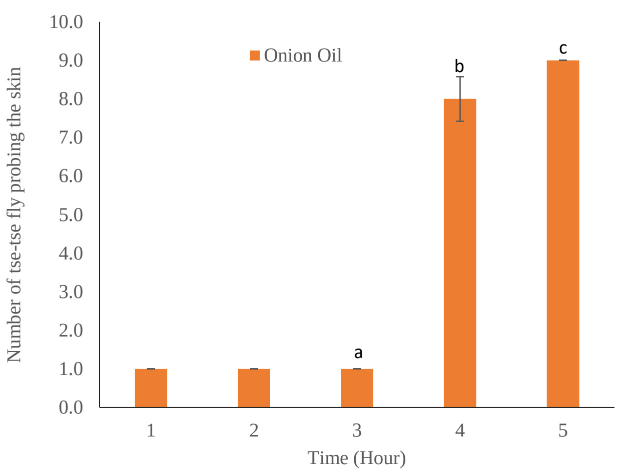

Figure 2b. Showing the mean number of *Glossina palpalis gambiensis* probing on skin after application of 75% onion oil. Means with different superscript letters ([a-c]) in different bars differ significantly (p<0.01).

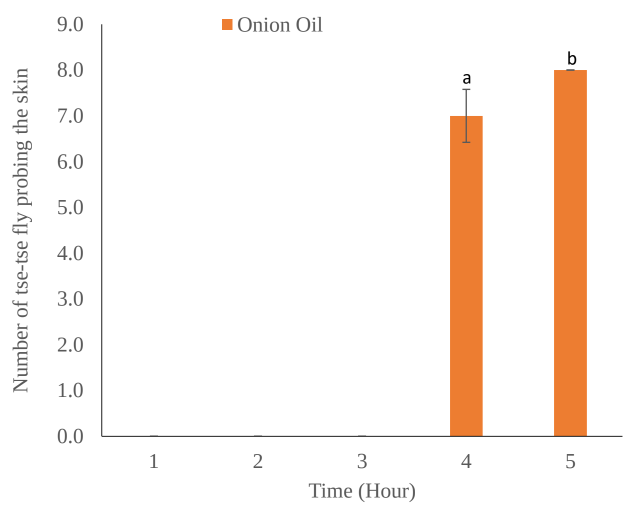

Figure 3b. Showing the mean number of *Glossina palpalis gambiensis* probing on skin after application of 8.75% onion oil. Means with different superscript letters ([a-b]) in different bars differ significantly (p<0.01).

At the 3rd hour post-application of 75% concentration of onion oil extract on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, onion oil performed significantly (p < 0.01) well at repelling *G. p. gambiensis* from landing with only 3.0±0.00 mean number of *G. p. gambiensis* landing on skin (Figure 2a).

At the 3rd hour post-application of 75% concentration of onion oil on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, onion oil performed significantly (p < 0.01) well at repelling *G. p. gambiensis* from probing with only 1.0±0.00 mean number of *G. p. gambiensis* probing on skin (Figure 2b).

The repellent efficacies of 50% and 75% concentrations of onion oil against the landing and probing of *G. p. gambiensis* were time-dependent. At 50% and 75% concentrations post-application on skin, onion oil provided 0 hour protection against the landing and probing of *G. p. gambiensis* (Figure 5a and Figure 5b).

Mean number of *Glossina palpalis gambiensis* landing, and probing on skin post-application of 87.5% and 100% concentrations of onion oil extract

At the 3rd hour post-application of 87.5% concentration of onion oil extract on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, onion oil performed

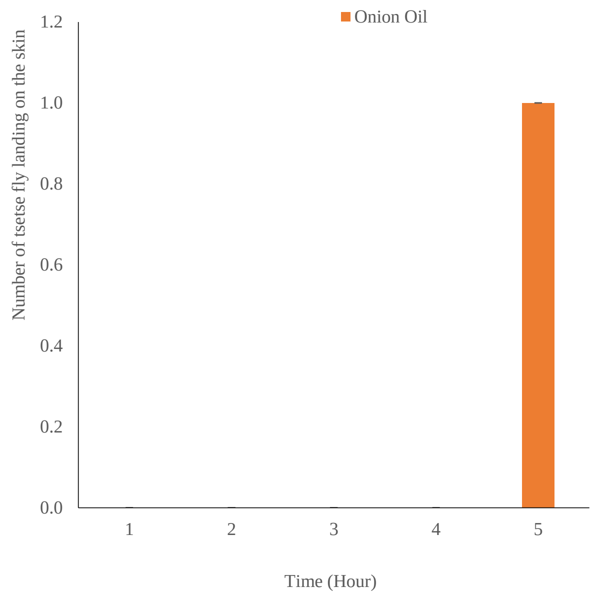

Figure 4a. Showing the mean number of *Glossina palpalis gambiensis* landing on skin after application of 100% onion oil (p<0.01).

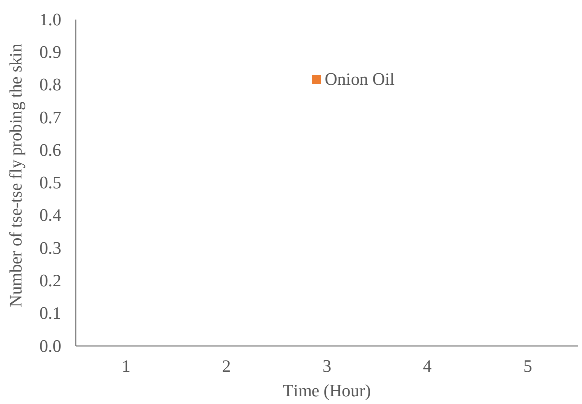

Figure 4b. Showing the mean number of *Glossina palpalis gambiensis* probing on skin after application of 100% onion oil (p<0.01).

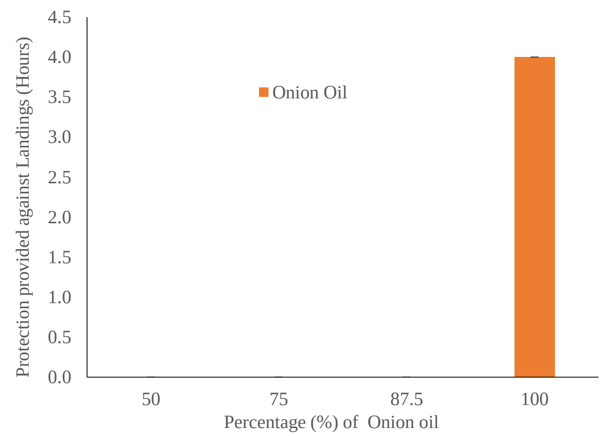

Figure 5a. Showing the mean protection provided by onion oil after application on skin, against the landings of *Glossina palpalis gambiensis* (p<0.01).

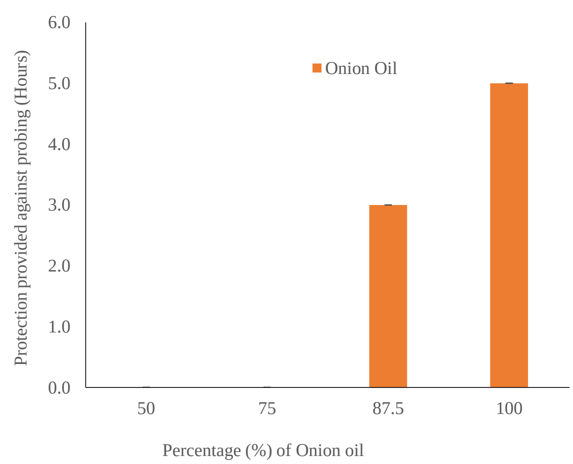

Figure 5b. Showing the mean protection provided by onion oil after application on skin, against the probing of *Glossina palpalis gambiensis* (p<0.01).

significantly (p < 0.01) well at repelling *G. p. gambiensis* from landing with only 1.0±0.00 mean number of *G. p. gambiensis* landing on skin (Figure 3a).

At the 1st, 2nd, and 3rd hours post-application of 87.5% concentration of onion oil on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, 0.0±0.00 mean numbers of *G. p. gambiensis* probed on skin.

At the 4th hour post-application of 87.5% concentration of onion oil on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, the efficacy of onion oil had waned (p < 0.01) with 7.0±0.58 mean number of *G. p. gambiensis* probing on skin (Figure 3b).

At 87.5% concentration post-application on skin, onion oil provided 0 hour protection against the landing of *G. p. gambiensis,* and 3 hours protection against the probing of *G. p. gambiensis* (Figure 5a and Figure 5b).

At the 1st, 2nd, 3rd, and 4th hour post-application of 100% concentration of onion oil extract on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, the mean number of *G. p. gambiensis* landing on skin was 0.0±0.00, 0.0±0.00, 0.0±0.00, and 0.0±0.00. At the 5th hour, the efficacy of 100% onion oil waned at repelling *G. p. gambiensis* from landing on skin (Figure 4a).

The mean numbers of *G. p. gambiensis* probing at 1st, 2nd, 3rd, 4th, and 5th hour post-application of 100% concentration of onion oil on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, were 0.0±0.00, 0.0±0.00, 0.0±0.00, 0.0±0.00, and 0.0±0.00 (Figure 4b).

Onion oil, at 100% concentration, exhibited efficacy (p < 0.01) at completely repelling *G. p. gambiensis* from probing the skin for the 5 hours test duration.

Onion oil at 100% concentration post-application on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, provided 4 hours protection against the landing of *G. p. gambiensis*, and 5 hours protection against the probing of *G. p. gambiensis* (Figure 5a and Figure 5b).

DISCUSSION

Organic chemical compounds such as thiosulfinates (allicin), vinyldithiins (2-vinyl-(4H)-1,3-dithiin, 3-vinyl-(4H)-1,2-dithiin), sulfides (diallyl disulfide) are reported to be largely responsible for the repellent properties of onion oil (Al-Snafi, 2013). The oil of onion released a characteristic odour, probably due to the sulfurous compounds identified in it. The gas chromatography and mass spectrometry analysis (GCMS) of the extracted oil of fresh onion bulb revealed important bioactive organic chemical compounds such as Dodecanoic acid, 9-Octadecenoic acid, n-Hexadecanoic acid, Tetradecanoic acid, Bromoacetic acid, 9-Oxabicyclo [6.1.0] nonane, Eicosene, and Carboxylic acid. This might have had powerful properties which have caused the onion oil to actively repel the tsetse flies. Sharaby *et al.* (2009) made similar observation.

The repellent efficacies of graded concentrations of onion oil post-application on skin exposed to starved laboratory-reared teneral *G. p. gambiensis*, were evaluated on the landing and the probing behaviour of *G. p. gambiensis*. The repellent efficacy of onion oil, and the protection in hours that onion oil provided against the landing, and the probing of *G. p. gambiensis* were concentration-dependent, and time-dependent. The number of *G. p. gambiensis* repelled from landing, and from probing, and the hours of protection from landing, and from probing given, increased with increasing concentration of onion oil, and with post-application time. Onion oil at 50%, 75%, and 87.5% concentrations post-application at one hour intervals performed less in efficacy than onion oil at 100% concentration post-application on skin in repelling *G. p. gambiensis* from landing and probing on skin. Onion oil at 100% concentration post-application on skin provided 4 hours protection against *G. p. gambiensis* landing, and 5-hours protection against *G. p. gambiensis* probing. Onion oil at 100% concentration created a great buffer against the probing of tsetse flies, providing 5 hours protection, making onion oil advantageous for the control of tsetse flies. This is in accordance with Pichersky and Gershenzon (2002), who reported that secondary metabolites of plants have evolved into repellents against hematophagous insects. Sharaby *et al.* (2009) also reported that the release of sulfur volatiles provided an important buffer against the Leek moth (*Acrolepiopsis assectella*), the mushroom fly (*Lycoriella ingenua*), and the potato tuber moth (*Phthorimaea opeculella*).

The repellent efficacy exhibited by onion oil against *G. p. gambiensis* landing and probing on human skin could be due to the bioactive potentials, such as insect enzyme-inhibition properties in the onion oil. Rattan (2010) stated that the mechanism of action of essential oils on the body of insects is due to the inhibition of acetylcholinesterase activity, which is a key enzyme responsible for nerve impulse transmission. Inhibition of acetylcholinesterase activity could weaken the flight of the tsetse flies, making them not able to actively seek their host. The onion oil applied to human fore-arm skin in this study caused no adverse effect to the human subjects who used it. Strickman *et al.* (2009) had reported that most of the plants used as repellents are also used for food flavouring or in the perfume industry, and this may explain why essential oils from plants are safer natural alternatives to synthetic insecticides.

Conclusion

In this study, onion oil extract was observed to be effective at repelling *G. p. gambiensis* from landing and probing on skin. This makes onions more advantageous for the common man, as onion bulbs are more abundantly available. Onion oil extract could be applied at a concentration of 100% at an interval of 5 hours on the skin, in a carefully planned husbandry practice, to protect humans and livestock from the bites of tsetse flies. Onion oil should be incorporated into the environmentally friendly methods of integrated control of tsetse fly, being advocated by the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) initiative to curb the transmission of trypanosomiasis. More research should be conducted on the oil of onion for more novel insights into its underlying mechanisms of action.

CONFLICT OF INTEREST

The author declare that they have no conflict of interest.

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